The importance of the SIBLING family of proteins on skeletal mineralisation and bone remodelling

The importance of the SIBLING family of proteins on skeletal mineralisation and bone remodelling

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Abstract

The small integrin-binding ligand N-linked glycoprotein (SIBLING) family consists of osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). These proteins share many structural characteristics and are primarily located in bone and dentin. Accumulating evidence has implicated the SIBLING proteins in matrix mineralization. Therefore, in this review we discuss the individual role that each of the SIBLING proteins has in this highly orchestrated process. In particular, we will emphasise how the nature and extent of their proteolytic processing and post-translational modification affects their functional role. Finally we establish what role the SIBLING proteins have in clinical disorders of hypophosphatemia and their potential therapeutic use.

Introduction

The skeleton is a highly intricate and complex organ that has a range of functions spanning from locomotion to ion homeostasis. It is structurally adapted to suit its function; strong and stiff to withstand loading, and yet light for movement and flexible to prevent fracture. The organic component of bone, termed the osteoid, comprises of an extracellular matrix (ECM) composed primarily of collagen type I together with several non-collagenous proteins (NCPs).

One such family of NCPs is the small integrin-binding ligand N-linked glycoprotein (SIBLING) family. This consists of osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). It is likely that this protein family arose from the sectrotry calcium-binding phosphoprotein (SCPP) family by gene duplication, due to their similar structural features (Kawasaki & Weiss 2006). Despite having little intrinsic sequence homology, the SIBLING family of proteins share some common characteristics (Fisher *et al.* 2001). All are located to a 375kb region on the human chromosome 4q21, and 5q in mouse, and have similar exon structures (Huq *et al.* 2005). The SIBLING proteins are principally expressed in bone and dentin, and are secreted into the ECM during osteoid formation and subsequent mineralisation.

All SIBLING proteins undergo similar post translational modifications (PTMs) such as phosphorylation and glycosylation, the extent of which is crucial in determining their function (Boskey *et al.* 2009). It has long been known that the SIBLING proteins have an Arg-Gly-Asp (RGD) sequence which facilitates cell attachment signaling by binding to cell surface integrins (Fisher *et al.* 2001). More recently, work by Peter Rowe and colleagues, primarily focused upon MEPE, has identified a new functional domain termed the ASARM peptide (acidic serine- and aspirate-rich motif) which is highly conserved between the SIBLING family members (Rowe *et al.* 2000; Rowe *et al.* 2004). This peptide is proving critical in the functional activity of the SIBLING proteins. The SIBLING proteins and their ASRAM motifs have individually been the subject of several excellent reviews, as are detailed later. In this review we focus upon the role in which each of the SIBLING

proteins have on skeletal matrix mineralisation and bone remodelling, as well as their clinical relevance in disorders of bone mineralisation.

Matrix mineralisation and bone remodelling

Endochondral ossification is a carefully orchestrated process attributed to the formation and postnatal linear growth of the long bones. It involves the replacement of a cartilage scaffold by mineralised bone. Integral to this process is the epiphyseal growth plate, a highly specialied cartilaginous structure derived from a mesenchyme precursor that is located between the head and the shaft of the bone. The growth plate consists of chondrocytes arranged in columns that parallel the axis of the bone surrounded by their extracellular matrix (ECM) (Mackie *et al.* 2008; Ballock & O'Keefe 2003) which is rich in collagens, proteoglycans and numerous other NCPs (Gentili & Cancedda 2009; Heinegard 2009). The chondrocytes of the growth plate sit in distinct cellular zones of maturation as are clearly visible (Fig. 1), and proceed through various stages of differentiation whilst maintaining their spatially fixed locations (Hunziker *et al.* 1987). It is the terminally differentiated hypertrophic chondrocyte that mineralizes its surrounding ECM, localized to the longitudinal septa of the growth plate (Castagnola *et al.* 1988).

Chondrocyte, as well as osteoblast, mineralization of the ECM is widely accepted to involve membrane-limited matrix vesicles (MVs) within which calcium (Ca^{2+}) and inorganic phosphate (P_i) accumulate to initiate the bi-phasic process of mineralization (Anderson 2003). When sufficient concentrations of both exist, calcium phosphate begins to precipitate to form HA crystals. This initial stage of mineralization is followed by the penetration of the MV trilaminar membrane by the HA crystals and the modulation of ECM composition, promoting the propagation of hydroxyapatite (HA) outside of the MVs (Anderson 2003; Anderson 1995; Wu *et al.* 2002; Golub 2011). Mineralization of the ECM is a tightly regulated process such that concentrations of Ca^{2+} and P_i are permissive for effective mineralization, and that the levels of mineralization inhibitors such as inorganic pyrophosphate (PP_i) are balanced. Extracellular PP_i is a well recognised and potent inhibitor of mineralization (Meyer 1984) that is regulated by alkaline phosphatase (ALP). In bone, ALP is a

ectoenzyme enzyme located on the cell membrane's outer surface of osteoblasts and chondrocytes, as well as on the membrane of their MVs (Anderson 1995). Classically, ALP was thought to generate the P_i required for HA formation, however it has since been shown to also hydrolyse PP_i thus achieving a ratio of P_i/PP_i permissive for HA crystal formation and growth (Fig. 2) (Hessle *et al.* 2002; Anderson 2003; Moss *et al.* 1967; Majeska & Wuthier 1975). PP_i inhibits the enzymatic activity of ALP (Addison *et al.* 2007) offering a feedback loop by which mineralization is mediated. Other regulators of ECM biomineralization include nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and the Ankylosis protein (ANK) which work in synergy to increase extracellular PP_i levels (Fig. 2). Whilst NPP1 ectoplasmically generates PP_i from nucleoside triphosphates , ANK mediates its intracellular to extracellular channelling (Ho *et al.* 2000; Hakim *et al.* 1984; Terkeltaub *et al.* 1994). Analysis of mice deficient in ALP function (*Akp2*$^{-/-}$) which are normally mineralized at birth has lead to the identification of other phosphatases such as PHOSPHO1. Since its discovery and characterisation, PHOSPHO1 has been proposed to play a crucial role in the accumulation of P_i within the MV and bone mineralisation (Stewart *et al.* 2006; Roberts *et al.* 2008; Roberts *et al.* 2007; MacRae *et al.* 2010; Houston *et al.* 2002). PHOSPHO1 has a non-redundant functional role during bone mineralisation and the ablation of both PHOSPHO1 and ALP results in the complete lack of bone mineralisation throughout the whole skeleton (Yadav et al. 2011)

Mineralization of the ECM not only facilitates the deposition of HA, but also enables vascular invasion; a significant phase in the development of the skeleton. Vascular invasion allows the infiltration of osteoclasts and differentiating osteoblasts which absorb the cartilaginous mineralized matrix and replace it with trabecular bone respectively. This synergistic process of resorption and formation continues throughout life, replacing approximately 10% of the adult skeleton every year (Frost 1990). Despite this, the net effect of osteoblasts and osteoclasts on bone mass is zero to ensure disorders such as osteoporosis or osteopetrosis do not occur. During bone resorption, osteoclasts adhere to the bone surface forming a tight connection and allowing efficient resorption through the release of acid and proteinases. Like bone formation, this is under tight control by a variety of

autocrine, paracrine and endocrine factors, and is thought to be primarily regulated by the terminally differentiated osteoblast, the osteocyte (Henriksen *et al.* 2009; Hill 1998; Manolagas 2000).

The SIBLING family of proteins

(1) MEPE

Matrix extracellular phosphoglycoprotein (MEPE) was originally identified as a substrate for PHEX (Phosphate regulating endopeptidase homolog, X-linked), a cell membrane associated glycoprotein. In bone, MEPE is primarily expressed by osteocytes, but *Mepe* mRNA expression has also been observed by chondrocytes and osteoblasts (Nampei *et al.* 2004)(REF). *Mepe* mRNA is detected as early as 2-days postpartum in the mouse skeleton (Lu *et al.* 2004). Several regulators of *Mepe* mRNA expression are documented in the literature. The addition of fibroblast growth factor 2 (FGF2) to osteoblasts down regulates *Mepe* mRNA levels in a dose dependent manner. The mechanism of action is part through the mitogen-activated protein kinase (MAPK) pathway (Zhang *et al.* 2004). Furthermore, osteoblasts stimulated with bone morphogenetic protein 2 (BMP2) also display a decreased *Mepe* mRNA expression level (Siggelkow *et al.* 2004).

The first evidence for a direct role of MEPE in bone mineralization came from the increased mRNA expression levels of *Mepe* seen during osteoblast matrix mineralization (Argiro *et al.* 2001; Petersen *et al.* 2000). The development of a MEPE null mouse further fuelled the proposed role for MEPE in mineralization. This mouse model had increased bone mass, with associated increased numbers and thickness of trabeculae. The mineral apposition rate (MAR) was dramatically increased as was the activity of MEPE null osteoblasts in culture (Gowen *et al.* 2003). Conversely, the overexpression of MEPE in mice, under the control of the coll1a1 promotor, leads to a growth and mineralization defect, due to a decrease in bone remodelling. The MEPE transgenic mice displayed wider epiphyseal growth plates, and expanded primary spongiosa and a significant decrease in the MAR (David *et al.* 2009).

Like the other SIBLING proteins, the activity of MEPE is dependent upon its state of cleavage and its phosphorylation. Recent work has identified the 2.2kDa ASARM peptide of MEPE as the central provider for the mineralization activity of MEPE. This ASARM peptide is highly conserved across

the SIBLING proteins and in MEPE, is located immediately downstream of a cathepsin B cleavage site (Rowe *et al.* 2000). The administration of the MEPE-ASARM peptide *in vitro* and *in vivo* can inhibit the uptake of P_i. This is likely through a decreased expression of the NPT2a renal phosphate transporter, or through the promotion of FGF23 expression, a potent inhibitor of Pi reabsorption (David *et al.* 2010; Dobbie *et al.* 2008; Shirley *et al.* 2010; Marks *et al.* 2008; Liu *et al.* 2007b; Martin *et al.* 2008). It has however been suggested that MEPE may have a direct effect on matrix mineralization outwith the supply and demand of P_i. The ASARM peptide of MEPE inhibits mineralization in osteoblasts by directly binding to HA crystals (Martin *et al.* 2008; Addison *et al.* 2008). This inhibitory effect has also been shown in growth plate chondrocytes (REF). Integral to this inhibitory effect is the post translational phosphorylation of the ASARM peptide at three serine residues. In osteoblasts it appears that without this phosphorylation, the ASARM peptide has no effect on mineralization (Martin *et al.* 2008; Addison *et al.* 2008) however in chondrocytes, it appears the non-phosphorylated ASARM peptide can promote matrix mineralization (REF). This is not the only evidence for a role for MEPE in the promotion of mineralization. Recently it has been shown that a truncated form of MEPE which has the ASARM peptide removed, can promote bone mineralization in culture and in mice (Sprowson *et al.* 2008). Furthermore, a mid-terminal fragment of MEPE (termed 'AC100') has been shown to enhance cell binding, through the stimulation of focal adhesion kinase and extracellular signal-regulated kinases (ERK) (Hayashibara *et al.* 2004). Taken together, these results highlight the importance of post-translational processing in determining the functional role of MEPE.

The interaction between MEPE and PHEX is well documented in the literature. PHEX plays a central role in the protection of MEPE from proteolytic cleavage by cathepsin B; it can bind to MEPE and prevent the release of the ASARM peptide (Guo *et al.* 2002). The *Hyp* mouse, a spontaneous *Phex* knockout model, has an increased expression of cathepsin D, an upstream activator of cathepsin B (Rowe *et al.* 2006). This therefore suggests that PHEX can alter the activation of cathepsin B, and therefore the cleavage of MEPE to the ASARM peptide. Furthermore, PHEX can bind to free

ASARM peptides therefore neutralizing their activity by sequestration and hydrolysis (Addison *et al.* 2008; Martin *et al.* 2008; Liu *et al.* 2007a).

MEPE transgenic mice display a decrease in ALP enzyme activity in both the growth plate and the primary spongiosa (David *et al.* 2009). *In vivo*, the addition of the phosphorylated ASARM peptide also reduced the number of ALP-positive cells in an osteoblast cell culture model (Martin *et al.* 2008). However, this remains controversial as normal ALP activity has been reported in osteoblasts treated with phosphorylated ASARM peptide (Addison *et al.* 2008). No difference was also observed in the metatarsal organ culture used to examine the effects of the ASARM peptide on chondrocyte mineralization. Intriguingly, this metatarsal model also showed that in chondrocytes, the phosphorylated ASARM peptide directly inhibits angiogenesis through an inhibition of VEGF and other markers associated with vascularisation (REF). Contradictory to this, in the MEPE overexpressing mouse vascularisation was increased, as was VEGF expression (David *et al.* 2009). Consonant with angiogenesis is the infiltration of osteoclasts for bone resorption. Interestingly, in mice administered with recombinant MEPE or transgenic for MEPE had a significant decrease in the numbers and activity of osteoclasts (David *et al.* 2009; Hayashibara *et al.* 2007).

(2) OPN

Osteoponin (OPN), also known as secreted phosphoprotein I (SPI), is a 34kDa protein, originally identified as the bridge between the cells and HA in the ECM of bone (Sodek *et al.* 2000). The protein and gene structure, as well as the localisation, of OPN are well described in several excellent reviews (Fisher *et al.* 2001; Sodek *et al.* 2000; Denhardt & Guo 1993). In bone, OPN is produced by osteoblasts and osteocytes (Zohar *et al.* 1997; Sodek *et al.* 1995), as well as osteoclasts (Dodds *et al.* 1995). It has also been localized to hypertrophic cartilage of the growth plate (Landis *et al.* 2003). This broad expression of OPN reflects its vast hormonal, cytokine and growth factor regulation.

Several studies have documented the inhibitory role of OPN in HA formation and growth (Hunter *et al.* 1994; Boskey *et al.* 1993). It has also been shown to inhibit mineralization in vascular smooth muscle cells (Jono *et al.* 2000; Wada *et al.* 1999). This inhibitory role for OPN is confirmed further by

analysis of the OPN knockout mouse which has increased mineral content and size, as shown by FT-IR analysis in two different lines of *Opn*$^{-/-}$ mice at two different ages (Boskey *et al.* 2002). More specifically, it has recently been shown that the ASARM peptide of OPN inhibits ECM matrix mineralization by binding to HA crystals (Addison *et al.* 2010). This study by Addison *et al* also showed that, like MEPE, the ability of the OPN-ASARM to inhibit mineralization is dependent upon its phosphorylation at specific serine residues (Addison *et al.* 2010). However, the phosphorylation of other serine residues outwith the ASARM motif can also inhibit HA formation (Pampena *et al.* 2004). This therefore emphasises the essentiality of phosphorylation in the functional capability of OPN to inhibit ECM mineralisation. This is further confirmed when examining the interaction between OPN, ALP and PP_i. Several studies have shown that ALP dephosphorylates OPN, thus preventing much of its inhibitory activity on HA formation and growth (Boskey *et al.* 1993; Jono *et al.* 2000; Hunter *et al.* 1994). Furthermore, PP_i directly upregulates OPN mRNA expression in osteoblasts, therefore the hydrolysis of PP_i by ALP will have a significant effect on the expression levels of OPN (Addison *et al.* 2007). This is in concordance with the *Enpp1* deficient mouse in which the PP_i deficiency brings about a deficiency in OPN (Johnson *et al.* 2003). This is also seen in the *Akp2* deficient mouse, and is further confirmed by the *Akp2*$^{-/-}$/*Opn*$^{-/-}$ mouse in which the hypomineralization seen in the *Akp2*$^{-/-}$ mouse alone is partially rescued (Harmey *et al.* 2006).

Although previous studies have implicated a phosphate dependent mechanism (Beck, Jr. *et al.* 2000; Beck, Jr. & Knecht 2003), work by Addison and colleagues has implicated the MAPK signaling pathways as responsible for the regulation of OPN by PP_i. Analysis of the *Opn*$^{-/-}$ mouse has also indicated a role for OPN in the function and activity of osteoclasts. An increase in osteoclast production by spleen cells from mice lacking OPN was observed (Rittling *et al.* 1998). This could be a compensatory mechanisms for the disabled motility and resorption activity in osteoclasts deficient in OPN (Chellaiah *et al.* 2003). Further studies have attempted to elucidate the precise role of OPN in bone resorption and have implicated CD44, a major cell-surface receptor for hyaluronate (Aruffo *et al.* 1990) and a receptor for OPN (Suzuki *et al.* 2002; Chellaiah *et al.* 2003).

(3) BSP

Bone sialoprotein (BSP) is a 70-80kDa protein for which its gene and protein structure have been extensively reviewed (Ganss *et al.* 1999). The localisation of BSP is unique to the SIBLING family of proteins as it is exclusively located to mineralized tissues such as bone, dentin and mineralizing cartilage (Chen *et al.* 1991; Bianco *et al.* 1991). In bone, it is expressed in abundance by osteoblasts, as well as by osteoclasts, osteocytes and chondrocytes (Gordon *et al.* 2007; Fisher & Fedarko 2003; Gordon *et al.* 2007).

During embryogenesis, BSP is first expressed at the onset of bone formation thus suggesting BSP to be a strong candidate for a role in HA nucleation (Chen *et al.* 1992). This certainly seems convincing as numerous studies have documented BSP to be involved in the initial formation of HA (Fisher *et al.* 2001; Harris *et al.* 2000; Tye *et al.* 2003; Wang *et al.* 2006). Indeed it has been shown that as little as 9nM BSP is required to nucleate HA and recently the overexpression of BSP in osteoblasts has been shown to enhance mineralization (Gordon *et al.* 2007; Hunter *et al.* 1996). Similarly, osteoblast cultures grown in the presence of an anti-BSP antibody exhibit reduced mineralization (Cooper *et al.* 1998; Mizuno *et al.* 2000). This nucleation potency is increased upon BSP binding to ????? suggesting a co-operative relationship between the two (Baht *et al.* 2008).

This nucleation has been reported to involve the membrane bound enzyme, ALP, where high. levels of ALP activity can promote the initiation of mineral deposition in the presence of BSP (Wang *et al.* 2006). This is further confirmed in BSP overexpressing cell cultures which have a higher ALP activity (Valverde *et al.* 2008). It is likely that, like the other SIBLING proteins, the function of BSP is highly dependent upon its PTM (Stubbs, III *et al.* 1997). In bone remodelling, BSP is crucial for the maintenance of osteoblast and osteoclast homeostasisc coupling. BSP increases osteoclastogenesis and therefore bone resorption (Valverde *et al.* 2005; Ross *et al.* 1993; Raynal *et al.* 1996). Furthermore, BSP expression in bone diseases characterised by excessive bone resorption, e.g. Pagets disease, is abnormally high (Valverde *et al.* 2008). This has been further examined in BSP transgenic

mice in which an uncoupling of bone formation and resorption resulted in an osteopenia-like phenotype (Valverde *et al.* 2008).

(4) DSPP

The role of dentin sialophosphoprotein (DSPP) in biomineralization has recently been reviewed (Prasad *et al.* 2010). Although originally thought to be exclusively expressed by dentin, DSPP is also expressed in bone, cementum and in non-mineralizing tissues including the lung and kidney (Verdelis *et al.* 2008; Alvares *et al.* 2006; Ogbureke & Fisher 2007; Qin *et al.* 2002; Baba *et al.* 2004).

DSPP is proteolytically processed to two fragments, dentin phosphoprotein (DPP) and dentin sialoprotein (DSP); both of which have important functions in mineralization. Interestingly, a third fragment called dentin glycoprotein (DGP) has been identified as being cleaved from the C-terminal end of DSP by MMP-2 and MMP-20 (Yamakoshi *et al.* 2005). It has been suggested that the proteolytic processing of DSPP to DPP, DSP and DGP is the activating stage in the mechanism of DSPP function (Prasad *et al.* 2010; Qin *et al.* 2004; Zhang *et al.* 2001). Analysis of the DSPP knockout mouse reveals defects in both dentin mineralization (Sreenath *et al.* 2003), as well as bone mineralization (Verdelis *et al.* 2008). In humans, a mutation in the *DSPP* gene results in dentinogenesis imperfecta characterized by dentin hypomineralization and significant tooth decay (Kim *et al.* 2005).

Of particular interest are the variations in the mineralization properties observed at different ages in the *Dspp*$^{-/-}$ mouse. This suggests that DSPP has roles in not only the initial mineralization of bone, but also in the remodelling of the skeleton. Various studies have shown DPP to be important in the formation and growth of HA as it has a strong affinity to Ca^{2+} when bound to collagen fibrils (Boskey *et al.* 1990; Saito *et al.* 1997; He *et al.* 2005). The phosphorylation of DPP is believed to be crucial to its function since removal of the phosphate groups results in a loss of its role in HA promotion (Saito *et al.* 1997). On the other hand, although DSP has been shown to be involved in the initiation of mineralization, it appears not to have a functional role in the maturation of the tissue (Suzuki *et al.* 2009). The mechanism by which DSPP regulates HA formation is thought to involve the canonical

BMP2 signaling pathway as BMP2 has been shown to increase DSPP mRNA expression via BMP-R Smads, Runx2 and DIx5 (Cho *et al.* 2010; Iohara *et al.* 2004; Chen *et al.* 2008).

(5) DMP1

Dentin matrix protein 1 (DMP1) was first cloned from dentin (George *et al.* 1993) and has since been identified in dentin, bone, and cementum (MacDougall *et al.* 1998), as well as other non-mineralized tissues (Sun *et al.* 2011). In bone, DMP1 is primarily expressed by osteocytes (Toyosawa *et al.* 2001) but also by osteoblasts (Feng *et al.* 2003) and hypertrophic chondrocytes (Fen *et al.* 2002). The first evidence for a role for DMP1 in biomineralization was its promotion of ECM mineralization in MC3T3 cells overexpressing DMP1 (Narayanan *et al.* 2001). The generation of a *Dmp1*-null mouse has further fuelled the potential role for DMP1 in bone mineralization. The mineral-matrix properties of the knockout mice were significantly lower when compared to their control counterparts (Ling *et al.* 2005). Furthermore, the DMP1 deficient mice displayed a severe defect in cartilage formation with a highly widened growth plate suggesting an impairment of mineralisation at the chondro-osseous junction. Indeed, this cartilage defect results in a phenotype resembling dwarfism with chondrodysplasia (Ye *et al.* 2005) and highlights the role of DMP1 in chondrocyte matrix mineralization. It has since been shown that the distorted growth plates seen in the DMP1 null mouse are in fact due to disorganisation as opposed to enlargement of what??? (Sun *et al.* 2010). Furthermore, this disorganisation can be recovered by crossing the DMP1 null mouse with transgenic mice expressing a mutant form of DMP1, which blocks the processing of mouse DMP1 *in vivo* (Sun *et al.* 2011). Like other SIBLING proteins, the proteolytically processing of DMP1 appears essential to its function and localization. In bone and dentin, DMP1 is processed to two fragments; one 37kDa fragment originating from the NH_2-terminal, and one 57kDa fragment originating from the COOH-terminal (Qin *et al.* 2003). In DMP1, it is the COOH-terminal fragment that contains the ASARM motif (Martin *et al.* 2008). The full length DMP1 is expressed at much lower levels (Huang *et al.* 2008) than its fragments which themselves have different localization patterns in bone (Maciejewska *et al.* 2008). In the growth plate, whilst the NH_2-terminal fragment is localized to the resting, proliferation and pre-hypertrophic zones, the COOH-terminal fragment is found in the calcification

front and ossification zone (Maciejewska *et al.* 2008). The localization of the COOH-terminal fragment is consistent with areas that are targets for the vascular invasion of the cartilage, a significant phase in matrix mineralization. DMP1 has been postulated to play a role in angiogenesis as treatment with DMP1 induced vascular endothelial cadherin (VE-cadherin), and inhibited the VEGFR-2 activity, therefore suggesting DMP1 to be an inhibitor of VEGF-induced angiogenesis (Pirotte *et al.* 2011). The direct role of DMP1 on HA formation is highly dependent upon its processing, and it's PTM. When phosphorylated, full length DMP1 has been shown to inhibit the formation and growth of HA (Tartaix *et al.* 2004), however, its dephosphorylated form and its two fragments are well established nucleators of HA formation (Tartaix *et al.* 2004; He *et al.* 2003; Gericke *et al.* 2010). Thus native DMP1 inhibits mineralization unless it becomes cleaved or dephosphorylated, in which case it initiates mineralization (Tartaix *et al.* 2004). The signaling pathways involved in DMP1 function have recently been investigated in osteoblasts. Wu *et al.,* 2011 showed DMP1, through the activation of the αvβ3 integrin, to activate the downstream effectors of the MAPK pathway, ERK and JNK (Wu *et al.* 2011). Concomitant to this is the stimulation of phosphorylated JNK translocation coupled with an up-regulation of phosphorylated c-jun activation (Wu *et al.* 2011). Furthermore, it has been shown that the internalization of DMP1 not only results in a release of stored Ca^{2+}, but also activates p38 MAP kinase (Eapen *et al.* 2011). DMP1 null mice have distinct abnormalities in the morphology and maturation of their osteocytes (Feng *et al.* 2006). The two DMP1 fragments also display differing localization patterns in osteocytes (Maciejewska *et al.* 2009), suggesting that osteocytes may play a critical role in ECM mineralization that involves DMP1. This is further supported by the stimulation of DMP1 expression in response to mechanical loading (Gluhak-Heinrich *et al.* 2007). Furthermore, the deletion of DMP1 leads to a dramatic increase in *Fgf23* mRNA expression in the osteocytes, likely due to the defects seen in osteoblast-osteocyte transition (Feng *et al.* 2006; Qin *et al.* 2007). FGF23, a hormone produced by osteoblasts and osteocytes, has allowed the definition of bone as an endocrine organ as it targets the kidney to regulate P_i homeostasis. This therefore suggests that DMP1 can control P_i levels, as is in concurrence with the hypophosphatemia observed in the DMP1 null mouse (Feng *et al.* 2006; Ye *et al.* 2005).

SIBLING ASARM peptides in mineralization diseases

Accumulating evidence has implicated the members of the SIBLING family of proteins in bone and mineralization diseases. Their varying involvements in the process of matrix mineralization make them potentially attractive candidates for therapeutic targets and therapies.

X-linked hypophosphatemic rickets (XLH) is the most common form of inherited rickets, characterised by defective bone and tooth mineralization, growth retardation, and defective renal re-absorption of P_i (Carpenter *et al.* 2011). Mutations in PHEX, have been associated with XLH in humans, and have led to the development of the *Hyp* mouse (Holm *et al.* 1997). Hypophosphatemia alone is insufficient to explain the bone defect seen in the *Hyp* mouse as correction of the hypophosphatemia failed to correct the mineralization defect observed (Ecarot *et al.* 1992; Rowe *et al.* 2006). Furthermore, when osteoblast cells from the *Hyp* mouse are grown in culture, they have a defective ECM production and thus reduced mineralization (Xiao *et al.* 1998). This therefore suggests that PHEX has multiple substrates which are involved in regulating mineralization directly. MEPE was first identified as a potential substrate for PHEX however *in vitro* studies have failed to demonstrate PHEX-dependent hydrolysis of MEPE (Guo *et al.* 2002). It has also been suggested that PHEX is likely responsible for the cleavage of DMP1 and DSPP, as it has a strong preference for cleaving bonds at the N-terminal of aspartyl residues found in these two SIBLING proteins (Qin *et al.* 2004). However analysis of the *Hyp* mouse indicated no differences in DMP1 and DSPP mRNA expression in comparison to their wild-type controls, suggesting that DMP1 and DSPP are in fact properly processed in the PHEX-deficient mouse (Zhang *et al.* 2010). In addition to this, there is an accumulation of SIBLING ASARM peptides in the *Hyp* mouse and patients with XLH (Bresler *et al.* 2004; Martin *et al.* 2008; Boukpessi *et al.* 2010) thus challenging the hypothesis that the SIBLING proteins are substrates for PHEX. Instead it appears that it is the ASARM peptide which PHEX digests (Addison *et al.* 200*,* 2010). This therefore provides compelling evidence for the ASARM hypothesis, as proposed by Peter Rowe, which describes the role of the SIBLING ASARM peptides, PHEX, and FGF23 in bone renal phosphate homeostasis, and therefore mineralization (Fig. 2) (Rowe 2004; David *et al.* 2010). This hypothesis can be used to explain numerous disorders of mineralization

including tumor-induced osteomalacia, autosomal-dominant hypophosphatemic rickets, and XLH. The potential for a therapeutic treatment is therefore high however further elucidation of the exact mechanisms involved is required.

Conclusions

The aim of this review is to present an overview of the role that each member of the SIBLING family of proteins has in matrix mineralization. The SIBLING proteins are principally found in bone and dentin, and are secreted into the ECM during its formation and subsequent mineralization. It is apparent that the functional role of the SIBLING proteins is highly dependent upon their state of cleavage and their post translational modification (Table 1). Furthermore, the identification of the ASARM motif which is present across the SIBLING proteins is proving critical in the functional activity of the SIBLING proteins. Much remains to be learnt regarding the *in vivo* role of the SIBLING protein and the ASARM peptide in bone disease. Future investigations should focus upon determining the interactions between the SIBLING proteins and the potential therapeutic use that they may have.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Reference List

Addison WN, Azari F, Sorensen ES, Kaartinen MT & McKee MD 2007 Pyrophosphate inhibits mineralization of osteoblast cultures by binding to mineral, up-regulating osteopontin, and inhibiting alkaline phosphatase activity. *J.Biol.Chem.* **282** 15872-15883.

Addison WN, Masica DL, Gray JJ & McKee MD 2010 Phosphorylation-dependent inhibition of mineralization by osteopontin ASARM peptides is regulated by PHEX cleavage. *J.Bone Miner.Res.* **25** 695-705.

Addison WN, Nakano Y, Loisel T, Crine P & McKee MD 2008 MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. *J.Bone Miner.Res.* **23** 1638-1649.

Alvares K, Kanwar YS & Veis A 2006 Expression and potential role of dentin phosphophoryn (DPP) in mouse embryonic tissues involved in epithelial-mesenchymal interactions and branching morphogenesis. *Dev.Dyn.* **235** 2980-2990.

Anderson HC 1995 Molecular biology of matrix vesicles. *Clin.Orthop.Relat Res.* 266-280.

Anderson HC 2003 Matrix vesicles and calcification. *Curr.Rheumatol.Rep.* **5** 222-226.

Argiro L, Desbarats M, Glorieux FH & Ecarot B 2001 Mepe, the gene encoding a tumor-secreted protein in oncogenic hypophosphatemic osteomalacia, is expressed in bone. *Genomics* **74** 342-351.

Aruffo A, Stamenkovic I, Melnick M, Underhill CB & Seed B 1990 CD44 is the principal cell surface receptor for hyaluronate. *Cell* **61** 1303-1313.

Baba O, Qin C, Brunn JC, Wygant JN, McIntyre BW & Butler WT 2004 Colocalization of dentin matrix protein 1 and dentin sialoprotein at late stages of rat molar development. *Matrix Biol.* **23** 371-379.

Baht GS, Hunter GK & Goldberg HA 2008 Bone sialoprotein-collagen interaction promotes hydroxyapatite nucleation. *Matrix Biol.* **27** 600-608.

Ballock RT & O'Keefe RJ 2003 The biology of the growth plate. *J.Bone Joint Surg.Am.* **85-A** 715-726.

Beck GR, Jr. & Knecht N 2003 Osteopontin regulation by inorganic phosphate is ERK1/2-, protein kinase C-, and proteasome-dependent. *J.Biol.Chem.* **278** 41921-41929.

Beck GR, Jr., Zerler B & Moran E 2000 Phosphate is a specific signal for induction of osteopontin gene expression. *Proc.Natl.Acad.Sci.U.S.A* **97** 8352-8357.

Bianco P, Fisher LW, Young MF, Termine JD & Robey PG 1991 Expression of bone sialoprotein (BSP) in developing human tissues. *Calcif.Tissue Int.* **49** 421-426.

Boskey AL, Chiang P, Fermanis A, Brown J, Taleb H, David V & Rowe PS 2009 MEPE's Diverse Effects on Mineralization. *Calcif.Tissue Int.*

Boskey AL, Maresca M, Doty S, Sabsay B & Veis A 1990 Concentration-dependent effects of dentin phosphophoryn in the regulation of in vitro hydroxyapatite formation and growth. *Bone Miner.* **11** 55-65.

Boskey AL, Maresca M, Ullrich W, Doty SB, Butler WT & Prince CW 1993 Osteopontin-hydroxyapatite interactions in vitro: inhibition of hydroxyapatite formation and growth in a gelatin-gel. *Bone Miner.* **22** 147-159.

Boskey AL, Spevak L, Paschalis E, Doty SB & McKee MD 2002 Osteopontin deficiency increases mineral content and mineral crystallinity in mouse bone. *Calcif.Tissue Int.* **71** 145-154.

Boukpessi T, Gaucher C, Leger T, Salmon B, Le FJ, Willig C, Rowe PS, Garabedian M, Meilhac O & Chaussain C 2010 Abnormal presence of the matrix extracellular phosphoglycoprotein-derived acidic serine- and aspartate-rich motif peptide in human hypophosphatemic dentin. *Am.J.Pathol.* **177** 803-812.

Bresler D, Bruder J, Mohnike K, Fraser WD & Rowe PS 2004 Serum MEPE-ASARM-peptides are elevated in X-linked rickets (HYP): implications for phosphaturia and rickets. *J.Endocrinol.* **183** R1-R9.

Carpenter TO, Imel EA, Holm IA, Jan de Beur SM & Insogna KL 2011 A clinician's guide to X-linked hypophosphatemia. *J.Bone Miner.Res.* **26** 1381-1388.

Castagnola P, Dozin B, Moro G & Cancedda R 1988 Changes in the expression of collagen genes show two stages in chondrocyte differentiation in vitro. *J.Cell Biol.* **106** 461-467.

Chellaiah MA, Kizer N, Biswas R, Alvarez U, Strauss-Schoenberger J, Rifas L, Rittling SR, Denhardt DT & Hruska KA 2003 Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. *Mol.Biol.Cell* **14** 173-189.

Chen J, Shapiro HS & Sodek J 1992 Development expression of bone sialoprotein mRNA in rat mineralized connective tissues. *J.Bone Miner.Res.* **7** 987-997.

Chen JK, Shapiro HS, Wrana JL, Reimers S, Heersche JN & Sodek J 1991 Localization of bone sialoprotein (BSP) expression to sites of mineralized tissue formation in fetal rat tissues by in situ hybridization. *Matrix* **11** 133-143.

Chen S, Gluhak-Heinrich J, Martinez M, Li T, Wu Y, Chuang HH, Chen L, Dong J, Gay I & MacDougall M 2008 Bone morphogenetic protein 2 mediates dentin sialophosphoprotein expression and odontoblast differentiation via NF-Y signaling. *J.Biol.Chem.* **283** 19359-19370.

Cho YD, Yoon WJ, Woo KM, Baek JH, Park JC & Ryoo HM 2010 The canonical BMP signaling pathway plays a crucial part in stimulation of dentin sialophosphoprotein expression by BMP-2. *J.Biol.Chem.* **285** 36369-36376.

Cooper LF, Yliheikkila PK, Felton DA & Whitson SW 1998 Spatiotemporal assessment of fetal bovine osteoblast culture differentiation indicates a role for BSP in promoting differentiation. *J.Bone Miner.Res.* **13** 620-632.

David V, Martin A, Hedge AM & Rowe PS 2009 Matrix extracellular phosphoglycoprotein (MEPE) is a new bone renal hormone and vascularization modulator. *Endocrinology* **150** 4012-4023.

David V, Martin AC, Hedge AM, Drezner MK & Rowe PS 2010 ASARM peptides: PHEX-dependent & independent regulation of serum phosphate. *Am.J.Physiol Renal Physiol*.

Denhardt DT & Guo X 1993 Osteopontin: a protein with diverse functions. *FASEB J.* **7** 1475-1482.

Dobbie H, Unwin RJ, Faria NJ & Shirley DG 2008 Matrix extracellular phosphoglycoprotein causes phosphaturia in rats by inhibiting tubular phosphate reabsorption. *Nephrol.Dial.Transplant.* **23** 730-733.

Dodds RA, Connor JR, James IE, Rykaczewski EL, Appelbaum E, Dul E & Gowen M 1995 Human osteoclasts, not osteoblasts, deposit osteopontin onto resorption surfaces: an in vitro and ex vivo study of remodeling bone. *J.Bone Miner.Res.* **10** 1666-1680.

Eapen A, Ramachandran A, Pratap J & George A 2011 Activation of the ERK1/2 Mitogen-Activated Protein Kinase Cascade by Dentin Matrix Protein 1 Promotes Osteoblast Differentiation. *Cells Tissues.Organs*.

Ecarot B, Glorieux FH, Desbarats M, Travers R & Labelle L 1992 Effect of dietary phosphate deprivation and supplementation of recipient mice on bone formation by transplanted cells from normal and X-linked hypophosphatemic mice. *J.Bone Miner.Res.* **7** 523-530.

Fen JQ, Zhang J, Dallas SL, Lu Y, Chen S, Tan X, Owen M, Harris SE & MacDougall M 2002 Dentin matrix protein 1, a target molecule for Cbfa1 in bone, is a unique bone marker gene. *J.Bone Miner.Res.* **17** 1822-1831.

Feng JQ, Huang H, Lu Y, Ye L, Xie Y, Tsutsui TW, Kunieda T, Castranio T, Scott G, Bonewald LB & Mishina Y 2003 The Dentin matrix protein 1 (Dmp1) is specifically expressed in mineralized, but not soft, tissues during development. *J.Dent.Res.* **82** 776-780.

Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF & White KE 2006 Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat.Genet.* **38** 1310-1315.

Fisher LW & Fedarko NS 2003 Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect.Tissue Res.* **44 Suppl 1** 33-40.

Fisher LW, Torchia DA, Fohr B, Young MF & Fedarko NS 2001 Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. *Biochem.Biophys.Res.Commun.* **280** 460-465.

Frost HM 1990 Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. *Anat.Rec.* **226** 403-413.

Ganss B, Kim RH & Sodek J 1999 Bone sialoprotein. *Crit Rev.Oral Biol.Med.* **10** 79-98.

Gentili C & Cancedda R 2009 Cartilage and bone extracellular matrix. *Curr.Pharm.Des* **15** 1334-1348.

George A, Sabsay B, Simonian PA & Veis A 1993 Characterization of a novel dentin matrix acidic phosphoprotein. Implications for induction of biomineralization. *J.Biol.Chem.* **268** 12624-12630.

Gericke A, Qin C, Sun Y, Redfern R, Redfern D, Fujimoto Y, Taleb H, Butler WT & Boskey AL 2010 Different forms of DMP1 play distinct roles in mineralization. *J.Dent.Res.* **89** 355-359.

Gluhak-Heinrich J, Pavlin D, Yang W, MacDougall M & Harris SE 2007 MEPE expression in osteocytes during orthodontic tooth movement. *Arch.Oral Biol.* **52** 684-690.

Golub EE 2011 Biomineralization and matrix vesicles in biology and pathology. *Semin.Immunopathol.* **33** 409-417.

Gordon JA, Tye CE, Sampaio AV, Underhill TM, Hunter GK & Goldberg HA 2007 Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization in vitro. *Bone* **41** 462-473.

Gowen LC, Petersen DN, Mansolf AL, Qi H, Stock JL, Tkalcevic GT, Simmons HA, Crawford DT, Chidsey-Frink KL, Ke HZ, McNeish JD & Brown TA 2003 Targeted disruption of the osteoblast/osteocyte factor 45 gene (OF45) results in increased bone formation and bone mass. *J.Biol.Chem.* **278** 1998-2007.

Guo R, Rowe PS, Liu S, Simpson LG, Xiao ZS & Quarles LD 2002 Inhibition of MEPE cleavage by Phex. *Biochem.Biophys.Res.Commun.* **297** 38-45.

Hakim FT, Cranley R, Brown KS, Eanes ED, Harne L & Oppenheim JJ 1984 Hereditary joint disorder in progressive ankylosis (ank/ank) mice. I. Association of calcium hydroxyapatite deposition with inflammatory arthropathy. *Arthritis Rheum.* **27** 1411-1420.

Harmey D, Johnson KA, Zelken J, Camacho NP, Hoylaerts MF, Noda M, Terkeltaub R & Millan JL 2006 Elevated skeletal osteopontin levels contribute to the hypophosphatasia phenotype in Akp2(-/-) mice. *J.Bone Miner.Res.* **21** 1377-1386.

Harris NL, Rattray KR, Tye CE, Underhill TM, Somerman MJ, D'Errico JA, Chambers AF, Hunter GK & Goldberg HA 2000 Functional analysis of bone sialoprotein: identification of the hydroxyapatite-nucleating and cell-binding domains by recombinant peptide expression and site-directed mutagenesis. *Bone* **27** 795-802.

Hayashibara T, Hiraga T, Sugita A, Wang L, Hata K, Ooshima T & Yoneda T 2007 Regulation of osteoclast differentiation and function by phosphate: potential role of osteoclasts in the skeletal abnormalities in hypophosphatemic conditions. *J.Bone Miner.Res.* **22** 1743-1751.

Hayashibara T, Hiraga T, Yi B, Nomizu M, Kumagai Y, Nishimura R & Yoneda T 2004 A synthetic peptide fragment of human MEPE stimulates new bone formation in vitro and in vivo. *J.Bone Miner.Res.* **19** 455-462.

He G, Dahl T, Veis A & George A 2003 Dentin matrix protein 1 initiates hydroxyapatite formation in vitro. *Connect.Tissue Res.* **44 Suppl 1** 240-245.

He G, Ramachandran A, Dahl T, George S, Schultz D, Cookson D, Veis A & George A 2005 Phosphorylation of phosphophoryn is crucial for its function as a mediator of biomineralization. *J.Biol.Chem.* **280** 33109-33114.

Heinegard D 2009 Proteoglycans and more--from molecules to biology. *Int.J.Exp.Pathol.* **90** 575-586.

Henriksen K, Neutzsky-Wulff AV, Bonewald LF & Karsdal MA 2009 Local communication on and within bone controls bone remodeling. *Bone* **44** 1026-1033.

Hessle L, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R & Millan JL 2002 Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proc.Natl.Acad.Sci.U.S.A* **99** 9445-9449.

Hill PA 1998 Bone remodelling. *Br.J.Orthod.* **25** 101-107.

Ho AM, Johnson MD & Kingsley DM 2000 Role of the mouse ank gene in control of tissue calcification and arthritis. *Science* **289** 265-270.

Holm IA, Huang X & Kunkel LM 1997 Mutational analysis of the PEX gene in patients with X-linked hypophosphatemic rickets. *Am.J.Hum.Genet.* **60** 790-797.

Houston B, Paton IR, Burt DW & Farquharson C 2002 Chromosomal localization of the chicken and mammalian orthologues of the orphan phosphatase PHOSPHO1 gene. *Anim Genet.* **33** 451-454.

Huang B, Maciejewska I, Sun Y, Peng T, Qin D, Lu Y, Bonewald L, Butler WT, Feng J & Qin C 2008 Identification of full-length dentin matrix protein 1 in dentin and bone. *Calcif.Tissue Int.* **82** 401-410.

Hunter GK, Hauschka PV, Poole AR, Rosenberg LC & Goldberg HA 1996 Nucleation and inhibition of hydroxyapatite formation by mineralized tissue proteins. *Biochem.J.* **317 (Pt 1)** 59-64.

Hunter GK, Kyle CL & Goldberg HA 1994 Modulation of crystal formation by bone phosphoproteins: structural specificity of the osteopontin-mediated inhibition of hydroxyapatite formation. *Biochem.J.* **300 (Pt 3)** 723-728.

Hunziker EB, Schenk RK & Cruz-Orive LM 1987 Quantitation of chondrocyte performance in growth-plate cartilage during longitudinal bone growth. *J.Bone Joint Surg.Am.* **69** 162-173.

Huq NL, Cross KJ, Ung M & Reynolds EC 2005 A review of protein structure and gene organisation for proteins associated with mineralised tissue and calcium phosphate stabilisation encoded on human chromosome 4. *Arch.Oral Biol.* **50** 599-609.

Iohara K, Nakashima M, Ito M, Ishikawa M, Nakasima A & Akamine A 2004 Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2. *J.Dent.Res.* **83** 590-595.

Johnson K, Goding J, van ED, Sali A, Hu SI, Farley D, Krug H, Hessle L, Millan JL & Terkeltaub R 2003 Linked deficiencies in extracellular PP(i) and osteopontin mediate pathologic calcification associated with defective PC-1 and ANK expression. *J.Bone Miner.Res.* **18** 994-1004.

Jono S, Peinado C & Giachelli CM 2000 Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. *J.Biol.Chem.* **275** 20197-20203.

Kawasaki K & Weiss KM 2006 Evolutionary genetics of vertebrate tissue mineralization: the origin and evolution of the secretory calcium-binding phosphoprotein family. *J.Exp.Zool.B Mol.Dev.Evol.* **306** 295-316.

Kim JW, Hu JC, Lee JI, Moon SK, Kim YJ, Jang KT, Lee SH, Kim CC, Hahn SH & Simmer JP 2005 Mutational hot spot in the DSPP gene causing dentinogenesis imperfecta type II. *Hum.Genet.* **116** 186-191.

Landis WJ, Jacquet R, Hillyer J & Zhang J 2003 Analysis of osteopontin in mouse growth plate cartilage by application of laser capture microdissection and RT-PCR. *Connect.Tissue Res.* **44 Suppl 1** 28-32.

Ling Y, Rios HF, Myers ER, Lu Y, Feng JQ & Boskey AL 2005 DMP1 depletion decreases bone mineralization in vivo: an FTIR imaging analysis. *J.Bone Miner.Res.* **20** 2169-2177.

Liu S, Rowe PS, Vierthaler L, Zhou J & Quarles LD 2007a Phosphorylated acidic serine-aspartate-rich MEPE-associated motif peptide from matrix extracellular phosphoglycoprotein inhibits phosphate regulating gene with homologies to endopeptidases on the X-chromosome enzyme activity. *J.Endocrinol.* **192** 261-267.

Liu S, Tang W, Zhou J, Vierthaler L & Quarles LD 2007b Distinct roles for intrinsic osteocyte abnormalities and systemic factors in regulation of FGF23 and bone mineralization in Hyp mice. *Am.J.Physiol Endocrinol.Metab* **293** E1636-E1644.

Lu C, Huang S, Miclau T, Helms JA & Colnot C 2004 Mepe is expressed during skeletal development and regeneration. *Histochem.Cell Biol.* **121** 493-499.

MacDougall M, Gu TT, Luan X, Simmons D & Chen J 1998 Identification of a novel isoform of mouse dentin matrix protein 1: spatial expression in mineralized tissues. *J.Bone Miner.Res.* **13** 422-431.

Maciejewska I, Cowan C, Svoboda K, Butler WT, D'Souza R & Qin C 2008 The NH2-terminal and COOH-terminal Fragments of Dentin Matrix Protein 1 (DMP1) Localize Differently in the Compartments of Dentin and Growth Plate of Bone. *J.Histochem.Cytochem.*

Maciejewska I, Qin D, Huang B, Sun Y, Mues G, Svoboda K, Bonewald L, Butler WT, Feng JQ & Qin C 2009 Distinct compartmentalization of dentin matrix protein 1 fragments in mineralized tissues and cells. *Cells Tissues.Organs* **189** 186-191.

Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS & Mirams M 2008 Endochondral ossification: how cartilage is converted into bone in the developing skeleton. *Int.J.Biochem.Cell Biol.* **40** 46-62.

MacRae VE, Davey MG, McTeir L, Narisawa S, Yadav MC, Millan JL & Farquharson C 2010 Inhibition of PHOSPHO1 activity results in impaired skeletal mineralization during limb development of the chick. *Bone* **46** 1146-1155.

Majeska RJ & Wuthier RE 1975 Studies on matrix vesicles isolated from chick epiphyseal cartilage. Association of pyrophosphatase and ATPase activities with alkaline phosphatase. *Biochim.Biophys.Acta* **391** 51-60.

Manolagas SC 2000 Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr.Rev.* **21** 115-137.

Marks J, Churchill LJ, Debnam ES & Unwin RJ 2008 Matrix extracellular phosphoglycoprotein inhibits phosphate transport. *J.Am.Soc.Nephrol.* **19** 2313-2320.

Martin A, David V, Laurence JS, Schwarz PM, Lafer EM, Hedge AM & Rowe PS 2008 Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are directly responsible for defective mineralization in HYP. *Endocrinology* **149** 1757-1772.

Meyer JL 1984 Can biological calcification occur in the presence of pyrophosphate? *Arch.Biochem.Biophys.* **231** 1-8.

Mizuno M, Imai T, Fujisawa R, Tani H & Kuboki Y 2000 Bone sialoprotein (BSP) is a crucial factor for the expression of osteoblastic phenotypes of bone marrow cells cultured on type I collagen matrix. *Calcif.Tissue Int.* **66** 388-396.

Moss DW, Eaton RH, Smith JK & Whitby LG 1967 Association of inorganic-pyrophosphatase activity with human alkaline-phosphatase preparations. *Biochem.J.* **102** 53-57.

Nampei A, Hashimoto J, Hayashida K, Tsuboi H, Shi K, Tsuji I, Miyashita H, Yamada T, Matsukawa N, Matsumoto M, Morimoto S, Ogihara T, Ochi T & Yoshikawa H 2004 Matrix extracellular phosphoglycoprotein (MEPE) is highly expressed in osteocytes in human bone. *J.Bone Miner.Metab* **22** 176-184.

Narayanan K, Srinivas R, Ramachandran A, Hao J, Quinn B & George A 2001 Differentiation of embryonic mesenchymal cells to odontoblast-like cells by overexpression of dentin matrix protein 1. *Proc.Natl.Acad.Sci.U.S.A* **98** 4516-4521.

Ogbureke KU & Fisher LW 2007 SIBLING expression patterns in duct epithelia reflect the degree of metabolic activity. *J.Histochem.Cytochem.* **55** 403-409.

Pampena DA, Robertson KA, Litvinova O, Lajoie G, Goldberg HA & Hunter GK 2004 Inhibition of hydroxyapatite formation by osteopontin phosphopeptides. *Biochem.J.* **378** 1083-1087.

Petersen DN, Tkalcevic GT, Mansolf AL, Rivera-Gonzalez R & Brown TA 2000 Identification of osteoblast/osteocyte factor 45 (OF45), a bone-specific cDNA encoding an RGD-containing protein that is highly expressed in osteoblasts and osteocytes. *J.Biol.Chem.* **275** 36172-36180.

Pirotte S, Lamour V, Lambert V, varez Gonzalez ML, Ormenese S, Noel A, Mottet D, Castronovo V & Bellahcene A 2011 Dentin matrix protein 1 induces membrane expression of VE-cadherin on endothelial cells and inhibits VEGF-induced angiogenesis by blocking VEGFR-2 phosphorylation. *Blood* **117** 2515-2526.

Prasad M, Butler WT & Qin C 2010 Dentin sialophosphoprotein in biomineralization. *Connect.Tissue Res.* **51** 404-417.

Qin C, Baba O & Butler WT 2004 Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Crit Rev.Oral Biol.Med.* **15** 126-136.

Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, Nagai N & Butler WT 2002 The expression of dentin sialophosphoprotein gene in bone. *J.Dent.Res.* **81** 392-394.

Qin C, Brunn JC, Cook RG, Orkiszewski RS, Malone JP, Veis A & Butler WT 2003 Evidence for the proteolytic processing of dentin matrix protein 1. Identification and characterization of processed fragments and cleavage sites. *J.Biol.Chem.* **278** 34700-34708.

Qin C, D'Souza R & Feng JQ 2007 Dentin matrix protein 1 (DMP1): new and important roles for biomineralization and phosphate homeostasis. *J.Dent.Res.* **86** 1134-1141.

Raynal C, Delmas PD & Chenu C 1996 Bone sialoprotein stimulates in vitro bone resorption. *Endocrinology* **137** 2347-2354.

Rittling SR, Matsumoto HN, McKee MD, Nanci A, An XR, Novick KE, Kowalski AJ, Noda M & Denhardt DT 1998 Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation in vitro. *J.Bone Miner.Res.* **13** 1101-1111.

Roberts S, Narisawa S, Harmey D, Millan JL & Farquharson C 2007 Functional involvement of PHOSPHO1 in matrix vesicle-mediated skeletal mineralization. *J.Bone Miner.Res.* **22** 617-627.

Roberts SJ, Owen HC & Farquharson C 2008 Identification of a novel splice variant of the haloacid dehalogenase: PHOSPHO1. *Biochem.Biophys.Res.Commun.* **371** 872-876.

Ross FP, Chappel J, Alvarez JI, Sander D, Butler WT, Farach-Carson MC, Mintz KA, Robey PG, Teitelbaum SL & Cheresh DA 1993 Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alpha v beta 3 potentiate bone resorption. *J.Biol.Chem.* **268** 9901-9907.

Rowe PS 2004 The wrickkened pathways of FGF23, MEPE and PHEX. *Crit Rev.Oral Biol.Med.* **15** 264-281.

Rowe PS, de Zoysa PA, Dong R, Wang HR, White KE, Econs MJ & Oudet CL 2000 MEPE, a new gene expressed in bone marrow and tumors causing osteomalacia. *Genomics* **67** 54-68.

Rowe PS, Kumagai Y, Gutierrez G, Garrett IR, Blacher R, Rosen D, Cundy J, Navvab S, Chen D, Drezner MK, Quarles LD & Mundy GR 2004 MEPE has the properties of an osteoblastic phosphatonin and minhibin. *Bone* **34** 303-319.

Rowe PS, Matsumoto N, Jo OD, Shih RN, Oconnor J, Roudier MP, Bain S, Liu S, Harrison J & Yanagawa N 2006 Correction of the mineralization defect in hyp mice treated with protease inhibitors CA074 and pepstatin. *Bone* **39** 773-786.

Saito T, Arsenault AL, Yamauchi M, Kuboki Y & Crenshaw MA 1997 Mineral induction by immobilized phosphoproteins. *Bone* **21** 305-311.

Shirley DG, Faria NJ, Unwin RJ & Dobbie H 2010 Direct micropuncture evidence that matrix extracellular phosphoglycoprotein inhibits proximal tubular phosphate reabsorption. *Nephrol.Dial.Transplant.* **25** 3191-3195.

Siggelkow H, Schmidt E, Hennies B & Hufner M 2004 Evidence of downregulation of matrix extracellular phosphoglycoprotein during terminal differentiation in human osteoblasts. *Bone* **35** 570-576.

Sodek J, Chen J, Nagata T, Kasugai S, Todescan R, Jr., Li IW & Kim RH 1995 Regulation of osteopontin expression in osteoblasts. *Ann.N.Y.Acad.Sci.* **760** 223-241.

Sodek J, Ganss B & McKee MD 2000 Osteopontin. *Crit Rev.Oral Biol.Med.* **11** 279-303.

Sprowson AP, McCaskie AW & Birch MA 2008 ASARM-truncated MEPE and AC-100 enhance osteogenesis by promoting osteoprogenitor adhesion. *J.Orthop.Res.* **26** 1256-1262.

Sreenath T, Thyagarajan T, Hall B, Longenecker G, D'Souza R, Hong S, Wright JT, MacDougall M, Sauk J & Kulkarni AB 2003 Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. *J.Biol.Chem.* **278** 24874-24880.

Stewart AJ, Roberts SJ, Seawright E, Davey MG, Fleming RH & Farquharson C 2006 The presence of PHOSPHO1 in matrix vesicles and its developmental expression prior to skeletal mineralization. *Bone* **39** 1000-1007.

Stubbs JT, III, Mintz KP, Eanes ED, Torchia DA & Fisher LW 1997 Characterization of native and recombinant bone sialoprotein: delineation of the mineral-binding and cell adhesion domains and structural analysis of the RGD domain. *J.Bone Miner.Res.* **12** 1210-1222.

Sun Y, Chen L, Ma S, Zhou J, Zhang H, Feng JQ & Qin C 2011 Roles of DMP1 processing in osteogenesis, dentinogenesis and chondrogenesis. *Cells Tissues.Organs* **194** 199-204.

Sun Y, Prasad M, Gao T, Wang X, Zhu Q, D'Souza R, Feng JQ & Qin C 2010 Failure to process dentin matrix protein 1 (DMP1) into fragments leads to its loss of function in osteogenesis. *J.Biol.Chem.*

Suzuki K, Zhu B, Rittling SR, Denhardt DT, Goldberg HA, McCulloch CA & Sodek J 2002 Colocalization of intracellular osteopontin with CD44 is associated with migration, cell fusion, and resorption in osteoclasts. *J.Bone Miner.Res.* **17** 1486-1497.

Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Terse A, Cho A, Kohler T, Muller R, Goldberg M & Kulkarni AB 2009 Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biol.* **28** 221-229.

Tartaix PH, Doulaverakis M, George A, Fisher LW, Butler WT, Qin C, Salih E, Tan M, Fujimoto Y, Spevak L & Boskey AL 2004 In vitro effects of dentin matrix protein-1 on hydroxyapatite formation provide insights into in vivo functions. *J.Biol.Chem.* **279** 18115-18120.

Terkeltaub R, Rosenbach M, Fong F & Goding J 1994 Causal link between nucleotide pyrophosphohydrolase overactivity and increased intracellular inorganic pyrophosphate generation demonstrated by transfection of cultured fibroblasts and osteoblasts with plasma cell membrane glycoprotein-1. Relevance to calcium pyrophosphate dihydrate deposition disease. *Arthritis Rheum.* **37** 934-941.

Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, Ijuhin N & Komori T 2001 Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. *J.Bone Miner.Res.* **16** 2017-2026.

Tye CE, Rattray KR, Warner KJ, Gordon JA, Sodek J, Hunter GK & Goldberg HA 2003 Delineation of the hydroxyapatite-nucleating domains of bone sialoprotein. *J.Biol.Chem.* **278** 7949-7955.

Valverde P, Tu Q & Chen J 2005 BSP and RANKL induce osteoclastogenesis and bone resorption synergistically. *J.Bone Miner.Res.* **20** 1669-1679.

Valverde P, Zhang J, Fix A, Zhu J, Ma W, Tu Q & Chen J 2008 Overexpression of bone sialoprotein leads to an uncoupling of bone formation and bone resorption in mice. *J.Bone Miner.Res.* **23** 1775-1788.

Verdelis K, Ling Y, Sreenath T, Haruyama N, MacDougall M, van der Meulen MC, Lukashova L, Spevak L, Kulkarni AB & Boskey AL 2008 DSPP effects on in vivo bone mineralization. *Bone* **43** 983-990.

Wada T, McKee MD, Steitz S & Giachelli CM 1999 Calcification of vascular smooth muscle cell cultures: inhibition by osteopontin. *Circ.Res.* **84** 166-178.

Wang J, Zhou HY, Salih E, Xu L, Wunderlich L, Gu X, Hofstaetter JG, Torres M & Glimcher MJ 2006 Site-specific in vivo calcification and osteogenesis stimulated by bone sialoprotein. *Calcif.Tissue Int.* **79** 179-189.

Wu H, Teng PN, Jayaraman T, Onishi S, Li J, Bannon L, Huang H, Close J & Sfeir C 2011 Dentin matrix protein 1 (DMP1) signals via cell surface integrin. *J.Biol.Chem.* **286** 29462-29469.

Wu LN, Genge BR, Kang MW, Arsenault AL & Wuthier RE 2002 Changes in phospholipid extractability and composition accompany mineralization of chicken growth plate cartilage matrix vesicles. *J.Biol.Chem.* **277** 5126-5133.

Xiao ZS, Crenshaw M, Guo R, Nesbitt T, Drezner MK & Quarles LD 1998 Intrinsic mineralization defect in Hyp mouse osteoblasts. *Am.J.Physiol* **275** E700-E708.

Yamakoshi Y, Hu JC, Fukae M, Zhang H & Simmer JP 2005 Dentin glycoprotein: the protein in the middle of the dentin sialophosphoprotein chimera. *J.Biol.Chem.* **280** 17472-17479.

Ye L, Mishina Y, Chen D, Huang H, Dallas SL, Dallas MR, Sivakumar P, Kunieda T, Tsutsui TW, Boskey A, Bonewald LF & Feng JQ 2005 Dmp1-deficient mice display severe defects in cartilage formation responsible for a chondrodysplasia-like phenotype. *J.Biol.Chem.* **280** 6197-6203.

Zhang B, Sun Y, Chen L, Guan C, Guo L & Qin C 2010 Expression and distribution of SIBLING proteins in the predentin/dentin and mandible of hyp mice. *Oral Dis.* **16** 453-464.

Zhang GX, Mizuno M, Tsuji K & Tamura M 2004 Regulation of mRNA expression of matrix extracellular phosphoglycoprotein (MEPE)/ osteoblast/osteocyte factor 45 (OF45) by fibroblast growth factor 2 in cultures of rat bone marrow-derived osteoblastic cells. *Endocrine* **24** 15-24.

Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, Wu G, Qiang B, Lo WH & Shen Y 2001 DSPP mutation in dentinogenesis imperfecta Shields type II. *Nat.Genet.* **27** 151-152.

Zohar R, Lee W, Arora P, Cheifetz S, McCulloch C & Sodek J 1997 Single cell analysis of intracellular osteopontin in osteogenic cultures of fetal rat calvarial cells. *J.Cell Physiol* **170** 88-100.